

## AMENDMENTS TO THE SPECIFICATION

Please enter the CRF version of the sequence listing concurrently submitted via EFS, a text file named 26774US\_CRF\_sequencelisting, created on March 11, 2010 and is 8,436 bytes in size.

Please INSERT the paragraph on page 1 of the specification, after the first paragraph, as follows:

### **SEQUENCE LISTING**

**The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 11, 2010, is named 26774US CRF sequencelisting.txt and is 8,436 bytes in size. In addition, the Sequence Listing filed concurrently herewith hereby replaces all previously filed paper copies of the Sequence Listing.**

Please REPLACE the paragraph beginning at page 6, line 9 of the specification as follows:

In a detailed embodiment contacting the carrier protein (CP) domain with the protein of interest (POI) further comprises synthesizing a CP domain -POI fusion protein to form a carrier protein (CP) domain -protein of interest (POI) complex. In a detailed aspect, the carrier protein (CP) domain further comprises an amino acid consensus sequence, [DEQGSTALMKRH]-[LIVMFYSTAC]-[GNQ]-[LIVMFYAG]-[DNEKHS]-S-[LIVMST]-{PCFY}-[STAGCPQLIVMF]-[LIVMATN]-[DENQGTAKRHLM]-[LIVMWSTA]-[LIVGSTACR]- (x2)-[LIVMFA] **(SEQ ID NO: 1)**.

Please REPLACE the paragraph beginning at page 10, line 1 of the specification as follows:

Figure 10 shows an application of the composition and method to tag fusion molecules with an SAFP-TAG. **Figure 10 discloses SEQ ID NOS 6-21, respectively, in order of appearance.**

Please REPLACE the paragraph beginning at page 10, line 24 of the specification as follows:

Figure 17 shows a system for combinatorial screening of carrier protein (CP) domains. **Figure 17 discloses SEQ ID NO: 22.**

Please REPLACE the paragraph beginning at page 20, line 24 of the specification as follows:

### **EXAMPLE 3**

#### **Tagging Heterologously Expressed Carrier Protein Domains**

Fluorescent tagging with derivatives in Figure 2 was repetitively conducted on proteins from crude cell lysate from recombinant *E. coli* BL21 cells expressing a carrier protein (i.e., VibB). Cell lysate was dialyzed to remove small molecules (<3 or <10 kDa), incubated with CoA-DYE and recombinant Sfp, and analyzed by SDS-PAGE. The outcome of this experiment is provided in Figure 11. When viewed under irradiation, recombinant VibB is visualized as a fluorescent band that was verified with two methods. First, standard Coomassie staining showed the fluorescent band to have the proper molecular weight when compared to molecular weight markers. Second, an identical gel was electrophoretically transferred to a polyvinylidene fluoride (PVDF) membrane, and the fluorescent band was excised from the membrane. This membrane piece was subjected to N-terminal amino acid sequencing by Edman degradation. The first 10 amino acids of the returned sequence, **MAIPKIASYP (SEQ ID NO: 2)**, mapped to the correct protein, VibB, when searched with BLAST against 1.4 million sequences in GenBank. Broad applicability of these techniques is anticipated for validating proper folding and modification ability of recombinant PK and NRP systems.

Please REPLACE the paragraph beginning at page 39, line 4 of the specification as follows:

## EXAMPLE 16

### PPTases can selectively transfer fluorescent CoA derivatives to carrier proteins

To investigate PPTase transfer of non-thioester CoA derivatives, Sfp was used for post-translational modification of known, heterologously expressed CP domains (Figure 11). As a first experiment, VibB was used. VibB is a small protein from the *Vibrio cholera* vibriobactin biosynthetic machinery that consists of a modular NRP synthase system. VibB contains only one carrier protein domain and as such is a perfect model system due to its small size and facile expression in *E. coli*. Cell lysate was collected from induced *E. coli* BL21 cells producing VibB from a pET24 expression vector. An aliquot of this lysate was incubated with CoA-BODIPY derivative and recombinant Sfp and analyzed by SDS-PAGE. When viewed under UV irradiation, recombinant VibB was visualized as a fluorescent band (Figure 11C). Coomassie staining of the gel confirmed the band to be fluorescently-tagged VibB (32.6 kD) (Figure 11C). Similarly, the formation of other fluorescent reporters were tested. Comparable labeling *apowas obtained after repetition of this experiment with Oregon Green® 488 maleimide (Table 1, 1b) and N-(7-dimethylamino-4-methylcoumarin-3-yl)maleimide (**1c**). Further proof was obtained by sequence analysis. A gel identical to Figure 11C was electrophoretically transferred to a polyvinylidene fluoride membrane, and the fluorescent band corresponding to VibB was excised from the membrane. The resulting piece was subjected to N-terminal amino acid sequencing by Edman degradation. Edman P., *Acta Chem. Scand.*, **4**: 283-293, 1950. The first 10 amino acids of the returned sequence, "MAIPKIASYP" (**SEQ ID NO: 2**), mapped to the correct protein, VibB, when searched with BLAST against 1.4 million sequences in GenBank. All three fluorescent analogs could be used to label, visualize, isolate, and sequence VibB.*

Please REPLACE the paragraphs beginning at page 51, line 12 of the specification as follows:

"Fused *apo*-CP homologs" refers to known CP domains having a consensus sequence within which the post-translational modification takes place. A fusion protein of the present invention can contain the consensus amino acid sequence or a homologous sequence thereof. The fusion partner can be as short as 13 amino acids, but it is considered a phosphopantetheinylation site if it has the consensus pattern. The consensus sequence is the following: [DEQGSTALMKRH]-[LIVMFYSTAC]-[GNQ]-[LIVMFYAG]-[DNEKHS]-S-[LIVMST]-{PCFY}-[STAGCPQLIVMF]-[LIVMATN]-[DENQGTAKRHLM]-[LIVMWSTA]-[LIVGSTACR]-x(2)-[LIVMFA] (**SEQ ID NO: 1**); wherein S is the pantetheine attachment site. *Concise Encyclopedia Biochemistry*, Second Edition, Walter de Gruyter, Berlin New-York (1988); Pugh E. L., et al., *J. Biol. Chem.* **240**: 4727-4733, 1965; Witkowski A., et al. *Eur. J. Biochem.* **198**: 571-579, 1991; <http://us.expasy.org/cgi-bin/nicedoc.pl?PDOC00012>.

The pattern rules are as follows. The PA (PAtern) lines contains the definition of a PROSITE pattern. The patterns are described using the following conventions: The standard IUPAC one-letter codes for the amino acids are used. The symbol 'x' is used for a position where any amino acid is accepted. Ambiguities are indicated by listing the acceptable amino acids for a given position, between square parentheses '[ ]'. For example: [ALT] stands for Ala or Leu or Thr. Ambiguities are also indicated by listing between a pair of curly brackets '{ }' the amino acids that are not accepted at a given position. For example: {AM} stands for any amino acid except Ala and Met. Each element in a pattern is separated from its neighbor by a '-'. Repetition of an element of the pattern can be indicated by following that element with a numerical value or a numerical range between parenthesis. For example: x(3) corresponds to x-x-x, x(2,4) corresponds to x-x or x-x-x or x-x-x-x. When a pattern is restricted to either the N- or C-terminal of a sequence, that pattern either starts with a '<'.

symbol or respectively ends with a '>' symbol. In some rare cases (e.g. PS00267 or PS00539), '>' can also occur inside square brackets for the C-terminal element. 'F-[GTV]-P-R-L-[G>]' **(SEQ ID NO: 3)** means that either 'F-[GTV]-P-R-L-G' **(SEQ ID NO: 4)** or 'F-[GTV]-P-R-L>' **(SEQ ID NO: 5)** are considered. A period ends the pattern.